this year; in fact, about 40% of the sampled fish were induced based upon elevated hepatic AHH activity that was inhibited by in vitro ANF. By contrast, none of the other teleost fish sampled had high AHH or ERF activity. In fact, in four of the species studied (eel, longhorn sculpin, hagfish and sea raven), hepatic AHH activity was below the level of detection in all fish studied. Consistent with this observation, ERF activities in these fish were also very low (3 to 11 pmol/min/mg protein). The remaining teleost species, the mackerel, had detectable hepatic AHH activity, but this activity was activated by in vitro ANF in each of the fish studied. This, coupled with the relatively low ERF activities, indicated that the hepatic MO system of these mackeral was not induced.

Over the last decade we have studied cytochrome P-450-dependent metabolism in more than a hundred little skates at MDIBL. Prior to this year we had never seen an untreated skate with high hepatic AHH and ERF activities, such as appears in Table 1. (The AHH and ERF activities for this skate were 1.2 units and 55 pmol/min/mg protein, respectively. Moreover, the AHH activity was inhibited to 0.59 units/min/mg protein by ANF). This single skate might have been exposed to a high local concentration of PAH-type inducing agents near MDIBL, or perhaps it was exposed elsewhere and migrated into the MDIBL vicinity. This would be consistent with our earlier studies in which we demonstrated that administration of β-NF or DBA to skates induces their hepatic MO system (Bend et al., Ann. N.Y. Acad. Sci. 298:505, 1977).

In conclusion, we have demonstrated that the winter flounder was the only teleost species sampled at MDIBL this summer in which a significant fraction of the population (about 40% of those sampled) had induced hepatic MO systems (i.e., high AHH activity inhibited by in vitro ANF). This may be related to the fact that flounder are demersal fish and spend almost their entire lives burrowed into sediment (Klein-MacPhee, NOAA Report, Nat. Mar. Fish. Service CIRC-414, 43 p., 1978), maximizing opportunity for interaction with lipophilic inducing chemicals of the PAH-type (e.g. PAH, CPB, dioxins, certain flavonoids). It appears that the other marine teleost species studied here (mackerel, longhorn sculpin and sea raven) are not exposed to sufficient concentrations of PAH-type inducers in their environment to affect their hepatic MO system, perhaps because expected routes of exposure to PAH-type chemicals are their food and the water column but not continuous exposure to contaminated sediment. As the hepatic MO system of all freshwater and marine species that have been adequately studied responds to PAH-type enzyme inducers, the low AHH and 7-ERF activities observed are almost certainly not due to the absence of the TCDD or PAH-cytosolic receptor (Poland et al., J. Biol. Chem. 251: 4936, 1976). In any event, the fact that these three species do not have induced hepatic MO systems makes them excellent marine fish in which to study the effect of PAH administration on the cytochrome P-450-dependent monooxygenase system.

BRAIN ESTROGEN BIOSYNTHESIS AND ESTROGEN CONJUGATING SYSTEMS IN THE SCULPIN (MYOXOCEPHALUS)

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Sex hormones, through their actions on target cells in the central nervous system (CNS), control sex behavior and gonadal functions. Although a commonly held dogma states that androgens and estrogens are, respectively, 'male' and 'female' hormones, they often share common actions which are independent of gender. In fact, each of the steroid classes and their specific receptors are present in both sexes. Moreover, testosterone and androstenedione are the immediate and obligatory precursors for estrogen biosynthesis (aromatization). Although aromatase is highest in the estrogen-secreting glandular tissues, low levels of activity in brain are essential for the full expression of certain central androgen actions. Thus, androgens not only have actions in their own right but also act via conversion to estrogen. As part of a phyletic survey, aromatase activity in the brain of the longhorn sculpin (Myoxocephalus octodecimspinosus) was found to be much higher than in mammals (190 vs 0.1-1.0 fmol/mg tissue) (Callard et al.,

Endocrinology 103:2283, 1978). Whereas aromatase in mammals is limited to regions having estrogen-binding cells (preoptic area, hypothalmus, limbic forebrain), in sculpin activity is also found outside the 'reproductive brain'. When the isolated head preparation is perfused with high specific activity ^{3}H-androgen, recovery of ^{3}H-estrogen products from discretely dissected brain regions conforms to the pattern of enzyme distribution as determined in vitro (anterior hypothalamus/preoptic area > medial telencephalon = lateral telencephalon > inferior lobes = thalamus = basal midbrain = cerebellum = medulla) (Callard et al., Gen. Comp. Endocrinal. 44:359, 1981). This indicates that in situ aromatization of circulating androgen is responsible for maintaining high concentrations of estrogen within the CNS. Such a mechanism would operate even in brain regions without conventional estrogen-concentrating systems (i.e., receptors) and may be essential where estrogen elicits short-latency, non-genomic responses.

In order to compare the neuroanatomic distribution of estrogen synthesized locally with that obtained directly from the circulation, the isolated sculpin head was perfused with ^{3}H-estradiol (5.6×10^{6} dpm). In marked contrast to results with androgen perfusion, no free estrone or estradiol was detectable in any brain region. Extraction of the spent perfusate failed to recover a substantial portion of the added substrate and also indicated that a large fraction had been converted to water-soluble metabolites (Table 1). These were identified as sulfates of estrone and

	(dpm x 10^{-6})					
	Total ^{3}H-Steroid		Estrone		Estradiol	
Fish #	Free	Polar	Free	Polar	Free	Polar
1	0.53	0.43	0.11	0.20	0.05	0.04
2	0.57	1.46	0.12	0.44	0.10	0.05
3	0.75	1.09	0.15	0.55	0.08	0.07

Table 1.--^{3}H-Steroid composition of medium following perfusion with ^{3}H-estradiol. Radioactivity was separated into total free (ether-soluble) and polar (water-soluble) fractions. Each was further analyzed by enzyme hydrolysis and thin-layer chromatography to estimate estrone and estradiol content. In the same experiments, no free estrogen was recovered from any of the brain regions analyzed (anterior hypothalamus, preoptic area, medial and lateral telencephalon, posterior hypothalamus, inferior lobes, thalamus, basal midrain, optic lobes, cerebellum, medulla).

estradiol. In order to locate the site of estrogen conjugation, minced tissues (200 mg) were incubated for 3 h in a balanced medium containing ^{3}H-estradiol (4 nM). Percentage conversion of added substrate (ether-soluble) to polar (water-soluble) products were considered a measure of conjugating activity. Tissues were ranked as follows: gill filaments (83.9%); liver (56.3%); kidney (52.6%); gut (53.7%); brain (28.87%); ovary (30.7%); muscle (20%). In a subsequent time-yield experiment using gill, ^{3}H-estradiol was utilized twice as efficiently as ^{3}H-testosterone (Callard, G.V. In: Reproductive Physiology of Fish, Pudoc, Wageningen, The Netherlands, p. 40, 1982). Mild acid hydrolysis (pH 5.0, 37° x 24 h) with or without Helix pomatia digestive juice (sulfatase/glucuronidase) or sulfatase-free glucuronidase indicated that most of the estrogen metabolites in the polar fraction were sulfates. In the presence of an appropriate cofactor (3'-phosphoadenosine-5'-phosphosulfate), the sulfotransferase activity in sculpin gill filaments was located in the cytosolic subfraction.

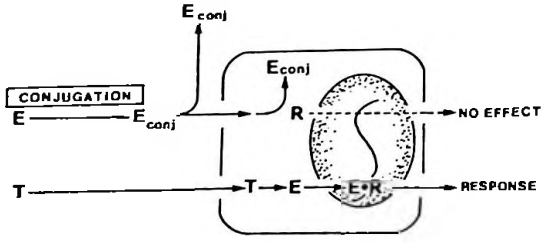

Figure 1.--Hypothetical scheme comparing access to CNS targets of circulating estrogen (E) versus estrogen synthesized in situ from circulating androgen (T). Conjugated estrogen ($\overline{E_{conj}}$) does not bind to receptors (R) and may also be excreted.

We conclude that in the isolated head preparation the gill effectively reduces free estrogen in the perfusate and prevents its accumulation in brain. Whether or not the gill also excretes conjugates is not known, although a substantial portion of the added substrate was lost somewhere in the perfusion system. Conjugated steroids have recently been identified as teleost pheromones and may enter the environment via this route. In contrast to estrogen, androgen apparently reaches the brain in amounts sufficient for aromatization. One explanation is that androgens are not as readily conjugated as estrogens. From studies in other vertebrates, it is known that conjugated estrogens are not acceptable ligands for receptor binding. It is likely, therefore, that central estrogen targets in the sculpin depend entirely on active hormone synthesized in close proximity to receptors. This hypothesis is depicted in Figure 1. An intriguing question is whether high brain aromatase activity in this species is a cause or a consequence of biological inactivation of estrogen in peripheral tissues (Supported by NSF PCM 78-23214/82-01975).

IDENTIFICATION OF AN ESTROGEN RECEPTOR IN THE TESTIS OF THE SHARK SQUALUS ACANTHIAS

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The shark Squalus acanthias is one of several non-mammalian vertebrates in which germ cells are topographically segregated within the testis according to their stage of development. This anatomical arrangement offers a unique opportunity to characterize biochemical changes occurring in conjunction with the cycle of the seminiferous epithelium. We previously reported distinct regional variations in the activities of several key steroidogenic enzymes leading to the formation of androgen and estrogen (Callard et al., The Bulletin 21:37, 1981). Since sex steroids have intratesticular sites of action in addition to their better known peripheral effects, we attempted to identify, characterize, and map the distribution pattern of an estrogen receptor in shark testis.

Using a dissecting microscope, testes were sectioned transversely and divided into four regions. Based on light microscopy, these were comprised of the following germ cell stages: I, spermatogonia; IIA, spermatocytes; IIB, round spermatids; III, mature spermatids. Epigonal tissues and mature spermatozoa collected from the vas deferens were used as control tissues. Cytosolic and nuclear extracts were prepared by differential centrifugation. All methods of receptor analysis are routinely used in this laboratory and have been described in detail (Mak et al., Biol. Reprod., in press, 1983). Unless otherwise stated, Zones I and IIA were used for characterizing the receptor. Nuclear extracts were labeled with ^{3}H-estradiol (5 nM) under exchange conditions (22° x 60 min, 4° x 15 min) to displace endogenous bound estrogen. Following separation of free and bound steroid on LH-20 columns, an estrogen binding component which could be displaced by 200-fold excess radioinert estradiol was detected. Comparison of displacement with estradiol and other steroids showed the following: estradiol (80%); diethylstilbestrol (57%); estrone (76%); estriol (61%); testosterone (38%); 5α-dihydrotestosterone (33%); progesterone (15%). When aliquots of nuclear extract were incubated with increasing concentrations of ^{3}H-estradiol (0.5 - 5.0 nM) $\pm$ 200-fold excess radioinert estradiol, specific binding approached saturation between 4 and 5 nM Figure 1). Scatchard analysis revealed a K_d of 1.53×10^{-9}M with a maximum binding capacity of 13.1 fmol per μg DNA (Figure 1). When nuclear extract labeled with ^{3}H-estradiol was analyzed by centrifugation on a high salt linear sucrose gradient (5-20%), a specific estrogen binding macromolecule having a sedimentation coefficient of 4.7 - 5S was detected. Measurement of binding capacity in different testicular regions revealed the following distribution (in fmol/ug DNA): Zone I (6.0); Zone IIA (3.5); Zone IIB (1.27); Zone III (0.69). No binding was found in epigonal tissue or in free spermatozoa.

Crude cytosol labeled by incubation with ^{3}H-estradiol $\pm$ 200-fold excess inert estradiol for 2 h at 4° was chromatographed on LH-20 and also on DNA-cellulose columns. DNA-cellulose affinity chromatography is useful for separating receptors (DNA-adhering) from low affinity, non-adhering estrogen binding molecules, usually serum contaminants. Crude cytosol was also subjected to a post-labeling method whereby samples chromatographed onto